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Research Article

Cymbidium kanran can deceptively attract *Apis cerana* for free pollination by releasing specialized volatile compounds

Huolin Luo[1*], Hanwen Xiao[1,2*], Xinchen Wu[1], Nannan Liu[1], Xinghui Chen[1], Dongjin Xiong[1], Weichang Huang[3], Boyun Yang[1]

1 *Jiangxi Key Laboratory of Plant Resources, School of Life Sciences, Nanchang University, Nanchang, China*
2 *College of Landscape Architecture, Nanjing Forestry University, Nanjing, 210037, China*
3 *Chenshan Botanical Garden, Shanghai 201602, China*
Corresponding author: Boyun Yang (yangboyun@163.com)

Abstract

Cymbidium kanran is classified as a second-level protected plant in China and is also listed in the World Genetic Conservation Plant Registry. Pollen flow is an important factor influencing the genetic structure of plant populations, holding significant relevance in the conservation of endangered plants. In this study, we present a comprehensive exploration of the pollination biology of *Cymbidium kanran*, encompassing investigations into its flowering phenology, breeding system, floral volatile components, and interactions with pollinating insects. The results showed that: 1) *C. kanran* exclusively relies on external pollination mechanisms, as automatic self-pollination or apomixis mechanisms are conspicuously absent. Consequently, the natural fruit set rate is significantly lower compared to artificial pollination, highlighting a pronounced pollination limitation. 2) *Apis cerana* emerges as the primary effective pollinating insect for *C. kanran*, adeptly carrying both pollinia and anther caps during the pollination process. Notably, *C. kanran* does not provide any rewards, such as nectar or edible pollen, to entice the pollinators. 3) Contrary to expectations, our glass cylinder experiment demonstrates that the flower color of *C. kanran* lacks significant attractiveness to pollinators ($p=0.1341>0.05$). However, the scent emitted by the flowers exhibits considerable allure ($p=0.0004<0.05$), despite *C. kanran* boasting one of the most diverse color variations within the Cymbidium genus. 4) Based on dynamic fluctuations in floral volatile components during different flowering stages, we hypothesize that hexanal, heptanal, octanal, 2-pentyl furan, 4-methyl-2-pentanone, and 1,4-cyclooctadiene may serve as pivotal volatile compounds responsible for attracting pollinators. This study establishes a robust scientific foundation for the conservation efforts concerning *C. kanran*, thereby facilitating the sustainable management and protection of its wild resources.

Key words: *Apis cerana*, breeding system, *Cymbidium kanran*, flowering phenology, pollination mechanism

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* These authors contributed equally to this work.

Introduction

Human activities have brought about a crisis in current biodiversity, reflected not only in a high rate of species extinction but also in the loss of interactions among species, leading to the emergence of many "zombie species" (Tylianakis et al. 2008; Traveset and Richardson 2014). Typically, researchers focus on species, proposing conservation strategies for rare or endangered species. However, such protection methods are inefficient, costly, and may not be sufficient to maintain the integrity of ecosystems (Laycock et al. 2009). Protecting species based on richness rather than functional importance may allocate substantial resources to specific species or even individual species, yielding low returns in terms of ecosystem integrity and functionality (Gotelli et al. 2012). Due to limited conservation management resources, researchers need to consider the cost-benefit ratio of conservation efforts. However, prioritizing species or habitats for protection is a complex task that requires considering the interspecific dependencies generated by interactions among species.

Ecological interactions among species provide services and functions for populations, communities, and ecosystems, constituting a crucial component of biodiversity (Kremen et al. 2007; Rader et al. 2016). Animal pollination is a common ecological interaction in terrestrial ecosystems, promoting the evolution and diversity of plants and pollinators (Wei et al. 2021). An estimated 85% of flowering plants and most crops directly consumed by humans rely on pollinators for reproduction (Ollerton et al. 2011). However, with habitat changes, climate change, and invasive species, the abundance and diversity of pollinators have sharply declined in recent years, potentially leading to more severe pollen limitation and reduced fruiting rates, affecting plant growth and community structure. Gomez et al. (2014) found that the decrease in pollinator diversity affects the growth of the generalist species *Erysimum mediohispanicum*. Biesmeijer et al. (2006) observed a correlated decline in the abundance of pollinators and their pollinated plants in the UK and the Netherlands, suggesting that the decrease in pollinator diversity may lead to a reduction in seed production and, consequently, impact the growth of plant populations for an extended period. Understanding how interactions between plants and pollinators affect species stability is crucial for predicting the consequences of species extinction (Bascompte 2009).

The Orchidaceae family stands out as one of the most highly evolved angiosperm groups, boasting approximately 800 genera and an astonishing 30,000 species distributed globally (Lu et al. 2019). Among these, *Cymbidium kanran*, a member of the Orchidaceae family, thrives as a perennial, terrestrial herbaceous plant within the moist soils of forested regions, typically at altitudes ranging from 400 to 2,400 meters. Its natural habitat spans across China, Japan, Korea, and neighboring regions. The allure of *C. kanran* is derived from its graceful foliage, diverse flower morphology, distinct fragrance, and substantial economic and ornamental value. However, these very attributes have led to the severe exploitation of its wild resources, exacerbated by the relentless encroachment upon its habitat, further jeopardizing the already precarious status of this orchid.

The floral structures of orchids exhibit remarkable specialization, featuring distinctive elements such as the specialized labellum, pollinia, and the gynandrium, formed through the fusion of stamens and pistils. The evolution of these specialized structures is thought to be the result of intricate interactions between orchids and their pollinators over their evolutionary history (Pramanik et al. 2020). To attract a broader array of pollinators, most orchids, mirroring other angiosperms, provide incentives such as nectar. However, approximately one-third of orchid species employ deceptive strategies, including sexual deception, food deception, and more, to accomplish pollination without offering any rewards to pollinating insects (Molnár et al. 2015).

Research conducted in Japan has shown that *C. kanran* can attract workers of *Apis cerana* japonica for pollination by emitting specific volatile compounds, despite these honeybees not receiving any food rewards during the pollination process (Tsuji and Kato 2010). Nevertheless, comprehensive data on the flowering phenology, reproductive biology, flower visitation behavior of pollinators, and the attractiveness of floral colors to pollinators—crucial elements in *C. kanran*'s pollination ecology—are still lacking. Additionally, the pollination strategy of the same plant species may adapt differently under diverse ecological conditions. Given that *A. cerana* japonica is less prevalent in China, it raises the intriguing question of how the pollination strategy of Chinese *C. kanran* may adjust. These intriguing inquiries have kindled our research interest. Consequently, this study delves into the flowering phenology, reproductive system, pollinators, and volatile compounds of *C. kanran*. Furthermore, it employs the Glass cylinder experiment to unravel the mechanisms behind pollinator attraction. Ultimately, this research aims to provide a scientific foundation for the conservation efforts directed towards Chinese *C. kanran*.

Materials and methods

Plant materials

The plant materials employed in this study included the wild population of *C. kanran* situated at Wuzhi Peak, Ganzhou City, Jiangxi Province (25°42'N, 114°40'E, elevation 632–648 m) for observations related to plant flowering phenology, breeding system experiments, insect pollination behavior, and detection of volatile components in flowers. Additionally, artificially cultivated *C. kanran* of five distinct varieties were selected for the glass cylinder experiment, namely "Lvbao" (green flowers with a pale green lip and purple spots), "Ziban" (purple-yellow flowers with a pale yellow lip), "Hongyu" (reddish-purple flowers with a pale green lip and purple-red spots), "Ehuang" (pale yellow-green flowers with a pale yellow lip and purple-red spots), and "Yincui" (silver-white sepals and petals with a pale green lip and purple spots).

Observation of flowering phenology

From October 2021 to January 2022, we meticulously observed 30 randomly labeled *C. kanran* plants at Wuzhi Peak, adhering to the guidelines established by Dafni (1992) for recording the flowering process. The flowering period of each individual

inflorescence, single flower, and the entire population was meticulously documented. Between December 1st and 8th, we continuously recorded the number of pollinia transferred in/out and the daily temperature over the course of a week. Fifteen fully bloomed flowers were chosen randomly, and the dimensions of the flower openings were measured using a vernier caliper with a precision of 0.001 mm.

Breeding system experiment

Thirty individuals of *C. kanran* were randomly selected from the wild population at Wuzhi Peak. To prevent the ingress of insects and foreign pollinia, breathable nylon bags were placed over the flower buds prior to flowering. On the first day of flowering, we removed the nylon bags and divided the individuals into six groups, each undergoing distinct treatments: (a) bagged (bag retained until flowers faded); (b) emasculation + bagged (removal of pollinia followed by bagging); (c) artificial self-pollination; (d) artificial geitonogamy; (e) artificial xenogamy; (f) Control (no bagging). Subsequently, we calculated the fruit set rate post-flowering and employed SPSS software for the analysis of differences among pollination methods.

Observation of pollinating insects and their behavior

During the peak flowering period, spanning from 8:00 to 17:00 daily, continuous recording of *C. kanran*'s pollinating insects and their behaviors was conducted at Wuzhi Peak. A camera (LUMIX, D1000) and a video camera (JVC, GZ-R10SAC) were employed for this purpose. This encompassed documenting their behavior before approaching the flower, the process of flower visitation, their landing and removal of pollinia, the duration of their stay on the flower, and the frequency of flower visitations. Additionally, we meticulously recorded the types and quantities of pollinating insects and preserved them as voucher specimens. From this group, 15 specimens were randomly selected for morphological characterization.

Glass cylinder experiment

To explore whether plants attract pollinators through olfactory or visual cues, an experiment was conducted employing three types of glass cylinders following the methodology outlined by Milet-Pinheiro et al. (2015): (a) a black cylinder with perforations, emitting odor without any visual cues (O cylinder); (b) a sealed, transparent cylinder, devoid of odor emission but with visual cues (V cylinder); and (c) a transparent cylinder with holes, facilitating both odor emission and visual cues (O/V cylinder) (Fig. 1). To facilitate odor emission from the cylinders with perforations, a coupled membrane pump (G12/01 EB; Rietschle Thomas, Germany) was used to circulate air through the cylinders at a rate of 1 L/min. Inflorescences of *C. kanran* were placed within O, V, and O/V cylinders to investigate the mode of attraction for pollinating insects, namely, whether the plant relied on olfactory or visual cues. Controls consisted of empty O, V, and O/V cylinders. Each type of glass cylinder was replicated three times, resulting in a total of 54 glass cylinders distributed within the wild population of *C. kanran*. Observations of *A. cerana* visitations were carried out between 8:00 AM and 4:00 PM on sunny days. The observations were conducted continuously over a span of three days, and the visitation frequency (average visits per hour) was calculated.

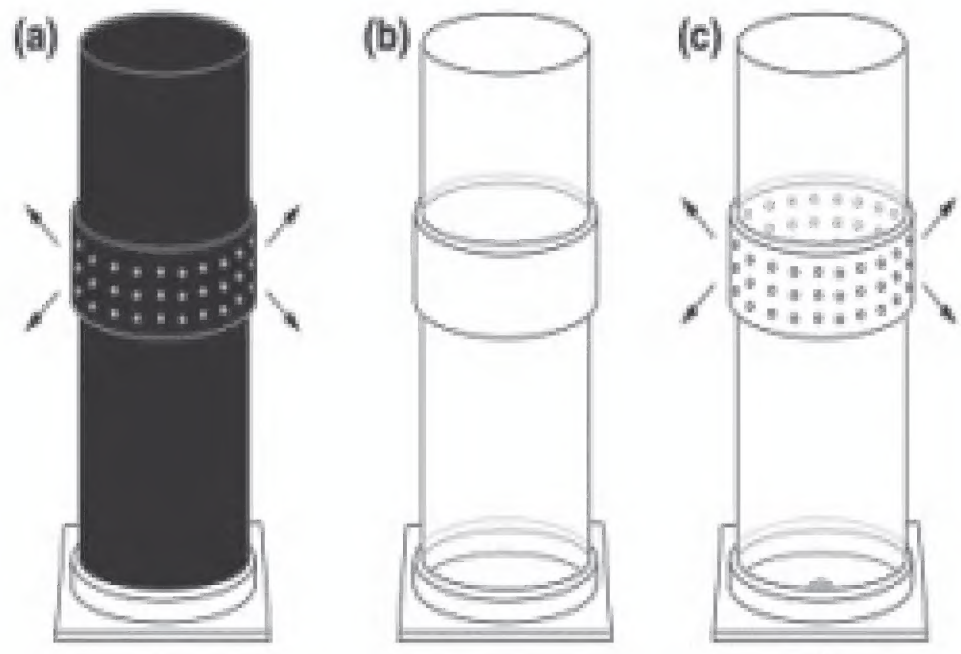

Figure 1. Glass cylinder that explores the behavior of insects **A** a black cylinder with holes (O cylinder) **B** a sealed and transparent cylinder (V cylinder) **C** a transparent cylinder with holes (O/V cylinder).

Detection of volatile components in flowers

The volatile components of *C. kanran* at different developmental stages (including the bud stage, blooming stage, and withering stage) and at various times of the day (8:00, 10:00, 12:00, 14:00, and 16:00) were analyzed using gas chromatography-mass spectrometry (Agilent, 6890 GC). Chemical components with a high degree of matching were selected through computer spectral library retrieval, and the samples were subjected to qualitative analysis following the protocol established by Wang et al. (2020).

Data analysis

Routine statistical analyses were carried out using IBM SPSS (version 19), while assessments of statistical significance were performed using GraphPad Prism 8.

Results

Temperature-dependent pollinia transfer efficiency

We observed a positive correlation between temperature and the efficiency of pollinia transfer in *C. kanran*. The flowering period of the *C. kanran* population spans approximately 120 days, with individual flowers remaining in bloom for an average of 33.6 days, and single inflorescences flowering for approximately 49.2 days. Continuous temperature monitoring, coupled with the quantification of pollinia movement over a week, revealed a pronounced correlation. Specifically, *C. kanran* exhibited extensive blooming on sunny days when daily maximum air temperatures exceeded 20 °C. Consequently, there was a significant increase in the quantity of pollinia movement, as illustrated in Fig. 2.

Insufficient media for the seed formation of *C. kanran*

Our investigation into the breeding system of *C. kanran* revealed a notable limitation in its natural seed formation potential. Both the “Bagged” and “Emasculation+Bagged” groups displayed a fruit set rate of 0, indicating the absence of

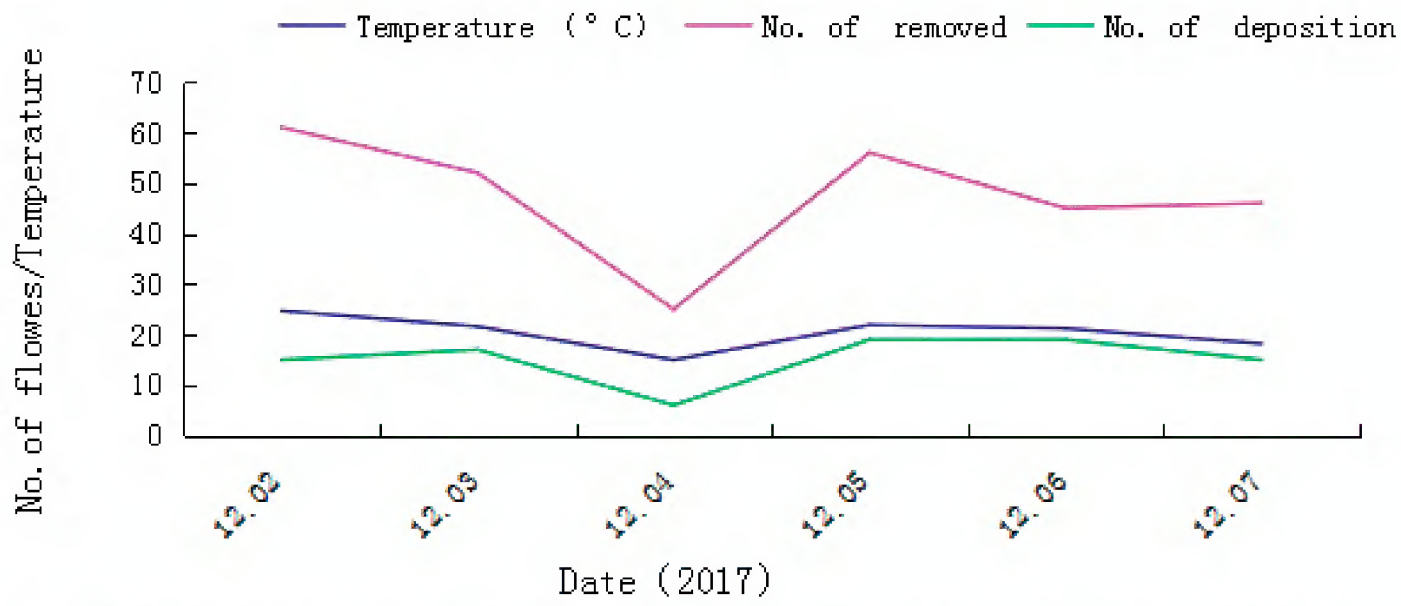

Figure 2. The relationship between temperature changes and the migration of pollinia.

automatic self-pollination and apomixis mechanisms in *C. kanran*. Therefore, successful seed formation in this species relies entirely on external pollination agents. In contrast, artificial self-pollination, artificial geitonogamy, and artificial xenogamy yielded fruit set rates exceeding 90%, as detailed in Table 1. Conversely, the control group exhibited a fruit set rate of less than 14%, underscoring the presence of substantial pollination limitations within the *C. kanran* population (Table 1).

Pollinating insects and their behavior

C. kanran attracts a diverse array of flower-visiting insects, including *A. cerana* (Apidae), *Chalcididae spp.*, *Syrphidae spp.*, and *Scutelleridae spp.* (Fig. 3A–D). Among these, only *A. cerana* possesses the ability to effectively carry pollinia. *A. cerana* typically visits between 1 to 9 flowers within a population and displays peak activity between 10:00–14:00 on sunny days. Our observations, conducted continuously for 7 consecutive days during the peak flowering period (8:00–17:00 each day), revealed that *A. cerana* visited *C. kanran* flowers a total of 151 times, with 53 of these visits involving the transportation of pollinia. When *A. cerana* carried pollinia, its average duration of stay on the flower was 51.28 seconds (± 6.29, n = 10). In contrast, when it visited a flower without carrying pollinia, its stay was significantly shorter, lasting 15.50 seconds on average (± 9.42, n = 10). The visiting behavior of *A. cerana* was observed to follow a specific sequence: it initially landed on the labellum and then entered the passage connecting the gynandrium and the labellum (Fig. 3E, F). As it approached the base of the gynandrium with its head, it became immobilized but continued to move its abdomen up and down until it exited (Fig. 3G). During the process of exiting, owing to the slightly larger thorax height of *A. cerana* compared to the passage's height, the pollinia on the gynandrium came into contact with the insect's back and adhered to it (Fig. 3H, Table 2). Subsequently, when *A. cerana* visited the next flower, it repeated similar movements, potentially transferring the pollinia into the gynandrium and thereby completing the pollination process.

Attraction of *A. cerana* by olfactory signals in *C. kanran*

Our findings suggest that *C. kanran* primarily attracts *A. cerana* through olfactory cues. Tukey's post-hoc test enabled us to classify the visitation frequency to the 18 distinct glass cylinders into two distinct levels. The first level displayed

Table 1. The fruit set rate in different pollination types.

Treatment	No.of flowers	Fruit set in orchid garden C (%)	Fruit set in orchid garden C (%)
bagged	30	0^{c}	0^{c}
Emasculation + bagged	30	0^{c}	0^{c}
Artificial self-pollination	30	93.33^{a}	96.67^{a}
Artificial geitonogamy	30	96.67^{a}	93.33^{a}
Artificial xenogamy	30	96.67^{a}	100^{a}
Control (unbagged)	30	11.18^{b}	13.33^{b}

Multiple comparisons were conducted by one-way ANOVA followed by Tukey's post-hoc test. Different lowercase letters indicate significant differences between groups ($P < 0.05$).

Table 2. Morphological characteristics of *C. kanran* and its pollinators.

	Sample size	Width of the passage/body width (mm)	Height of the passage/height of the thorax (mm)
C. kanran	15	8.982±0.812	3.474±0.176
A. cerana	15	6.010±0.290	3.592±0.108

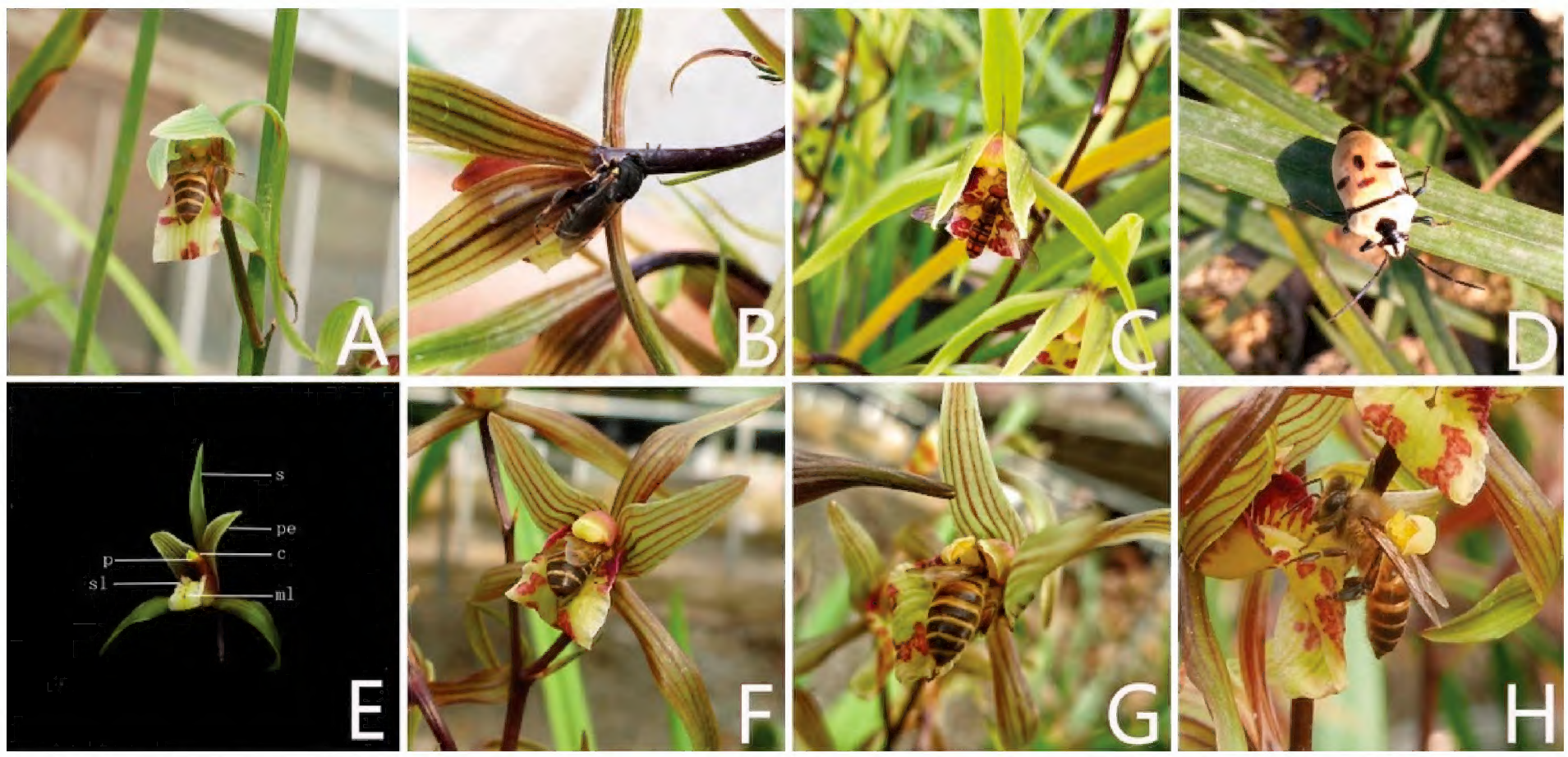

Figure 3. Visiting insects and pollinators of *C.kanran* **A** *A.cerana* visits flower **B** *Chalcididae* spp. visits flower **C** *Syrphidae* spp. visits flower **D** *Liscutelleridae* spp. visits flower **E** the flowers of *C.kanran*. c, column; ml, middle lobe; p, pollinium; pe, petals **F** *A.cerana* enters the passage connecting the gynandrium and the labellum **G** the *A.cerana* exits the passage **H** the back of *A.cerana* is already stuck with pollinium.

low visitation frequency and encompassed O-CK (olfactory-signal glass cylinders without inflorescence), V-CK (visual-signal glass cylinders without inflorescence), O/V-CK (combined-signal glass cylinders without inflorescence), and V-inflorescence (visual-signal glass cylinders with inflorescence of different varieties). In contrast, the second level demonstrated high visitation frequency and included O-inflorescence (olfactory-signal glass cylinders with inflorescence of different varieties) and O/V-inflorescence. Statistical analysis revealed that the visitation

frequency in the first four groups was significantly lower compared to the last two groups. Importantly, no significant differences were detected among the visitation frequencies within the first four groups or the last two groups. Furthermore, our observations indicated no significant disparity in bee attraction between inflorescences of different colors placed within the same glass cylinder (Table 3).

Candidate volatile components for pollinator attraction

The composition of volatile aroma compounds in *C. kanran* exhibited significant variations across different flowering stages. During the bud stage, the predominant volatile components included cyclobutanol, pentanol, 4-methyl-2-pentanone, and hexanal, constituting 20.86%, 14.38%, 5.14%, and 59.62% of the total, respectively. In the peak flowering stage, the primary components were pentanal, hexanal, 2-pentyl furan, l-Alanine, N-(1-oxopentyl)-, methyl ester, 4-methyl-2-pentanone, cyclopropane, 1,1-dimethyl-, and 1,4-cyclooctadiene, accounting for 19.09%, 66.47%, 0.85%, 7.67%, 3.00%, 1.49%, and 1.43%, respectively. During the withering stage, the main components comprised pentanal, hexanal, heptanal, 2-pentyl furan, octanal, 2-methylbutanal, isovaleraldehyde, nonanal, and 3-methyl-2-butenal, constituting 12.96%, 69.35%, 1.76%, 6.32%, 0.77%, 1.90%, 2.83%, 0.19%, and 0.81% of the total, respectively (Fig. 4).

Table 3. Frequency of pollinating insects visiting C. kanran based on a glass cylinder experiment.

Treatment	Visiting frequency	Treatment	Visiting frequency	Treatment	Visiting frequency
O-CK	0.00[b]	V-CK	0.04[b]	O/V-CK	0.00[b]
O-“Lvbao”	1.75a	V-“Lvbao”	0.00[b]	O/V-“Lvbao”	1.97[a]
O-“Ziban”	1.56[a]	V--“Ziban”	0.00[b]	O/V--“Ziban”	1.36[a]
O-“Hongyu”	1.48[a]	V-“Hongyu”	0.00[b]	O/V-“Hongyu”	1.28[a]
O-“Ehuang”	1.25[a]	V-“Ehuang”	0.00[b]	O/V-“Ehuang”	1.50[a]
O-“Yincui”	1.44[a]	V-“Yincui”	0.00[b]	O/V-Yincui”	1.67[a]

O: olfactory-signal glass cylinder; V: visual-signal glass cylinder; O/V: combined-signal glass cylinder; CK: no inflorescence placed in the glass cylinder; Green: C. kanran "Green Treasure" (green flowers with labellum in light green and purple spots); Purple: C. kanran "Purple Spot" (purple and yellow flowers with labellum in light yellow); Red: C. kanran "Red Jade" (red-purple flowers with labellum in light green and purple-red spots); Yellow: C. kanran "Goose Yellow" (pale yellow-green flowers with labellum in light yellow and purple-red spots); Silvery white: C. kanran "Silver Jade" (the outer sepals and petals are silver-white, and the labellum is light green with purple spots). Multiple comparisons were conducted by one-way ANOVA followed by Tukey's post-hoc test. Different lowercase letters indicate significant differences between groups ($P < 0.05$).

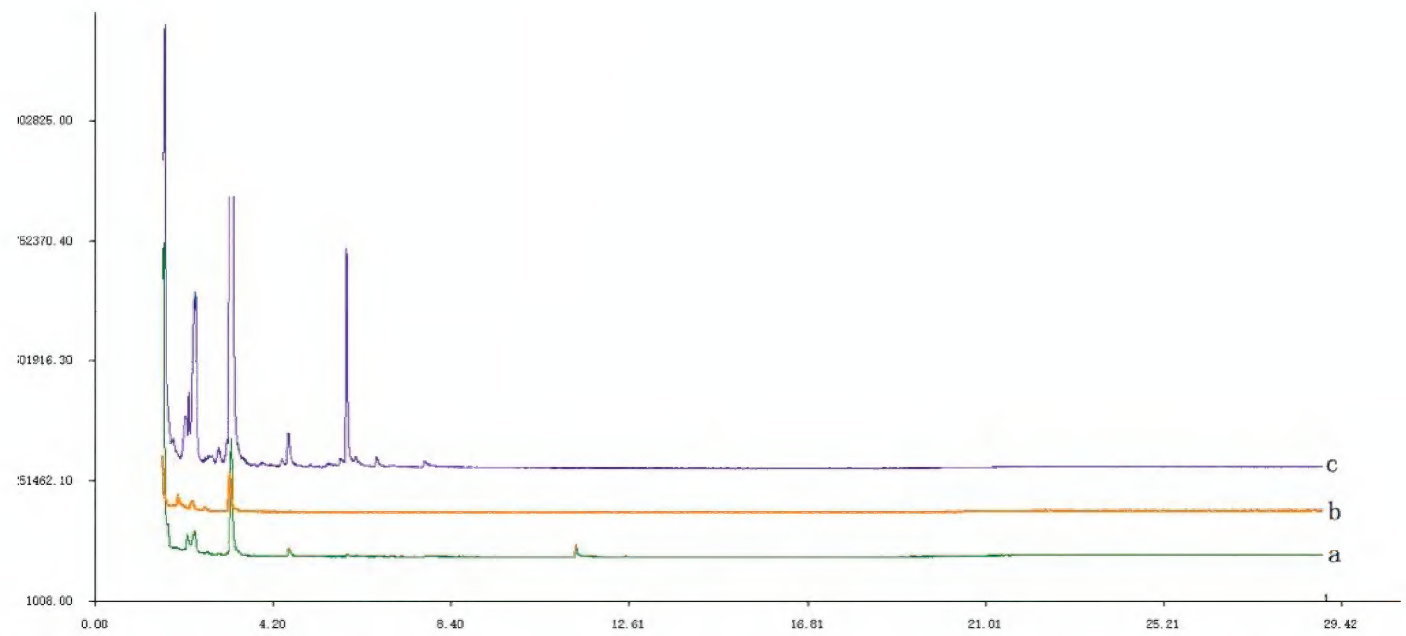

Figure 4. Total ion chromatograms of volatile components from flowers at different stages **a** bud stage **b** full bloom stage **c** withering stage.

Moreover, our analysis of volatile components at different times of the day (8:00, 10:00, 12:00, 14:00, and 16:00) during the full bloom stage revealed distinct patterns. At 8:00, the volatile components primarily comprised cyclobutanols, pentanols, hexanals, heptanals, 2-pentyl furan, and 3-octyne, representing 9.1%, 15.6%, 66.14%, 4.27%, 0.81%, and 4.09% of the total, respectively. At 10:00, the main volatile components were pentanals, hexanals, heptanals, 2-pentyl furan, and (±)-3-hydroxy-r-citronellal, accounting for 17%, 72.25%, 2.69%, 1.43%, and 4.35% of the total, respectively. At 12:00, the primary volatile components included pentanals, hexanals, 2-pentyl furan, l-Alanine, N-(1-oxopentyl)-, methyl ester, 4-methyl-2-pentanone, cyclopropane, 1,1-dimethyl-, and 1,4-cyclooctadiene, constituting 19.09%, 66.47%, 0.85%, 7.67%, 3%, 1.49%, and 1.43% of the total, respectively. At 14:00, the volatile components primarily consisted of pentanals, hexanals, heptanals, 2-pentyl furan, 4-methyl-2-pentanone, 1,4-cyclooctadiene, hexamethylcyclotrisiloxane, and octanals, making up 18.96%, 62.22%, 4.6%, 1.19%, 1.52%, 7.29%, 2.37%, and 1.84% of the total, respectively. Finally, at 16:00, the dominant volatile components were pentanals, hexanals, heptanals, and 2-pentyl furan, accounting for 17.19%, 73.74%, 4.87%, and 1.45% of the total, respectively (Fig. 5).

The combined analysis of Figs 5, 6 highlights several notable trends. Pentanals and hexanals consistently displayed relatively high contents throughout the entire flowering period. In contrast, heptanals and 2-pentyl furan were detected predominantly during the peak flowering period and were absent during the budding stage. Notably, 4-methyl-2-pentanone and 1,4-cyclooctadiene exhibited their highest abundance during the period when flower visitations by insects were most frequent (between 12:00 and 14:00), as depicted in Fig. 6.

Discussion

Deceptive pollination strategy in *C. kanran*

Our study reveals that *C. kanran* employs a deceptive pollination strategy to attract *A. cerana* for pollination. The comprehensive characteristics of the flower, the pollination mechanism, and the behavior of the pollinators are intricately linked to the fitness and reproductive success of the plant (Zhang et al. 2021).

C. kanran exhibits a unique set of traits that contribute to its deceptive pollination strategy. It blooms from October to January of the following year, producing numerous large, strongly scented flowers with an extended flowering period. Remarkably, each individual flower remains in bloom for up to 33 days. This prolonged flowering duration serves a crucial function by mitigating the adverse effects of low temperatures and potentially limited insect populations, thereby increasing the likelihood of successful pollination.

While mutualistic relationships between plants and pollinating insects are common in nature, characterized by the provision of various rewards such as nectar, oils, or lipids to attract pollinators (Burger et al. 2010; Kaiser-Bunbury et al. 2017; Wong et al. 2017), *C. kanran* stands apart. Unlike many other orchids and flowering plants, we did not observe any form of food reward for pollinators within the *C. kanran* flower. Furthermore, the flower's morphology and the behavior of the pollinators do not suggest the provision of functions such as breeding grounds or shelters.

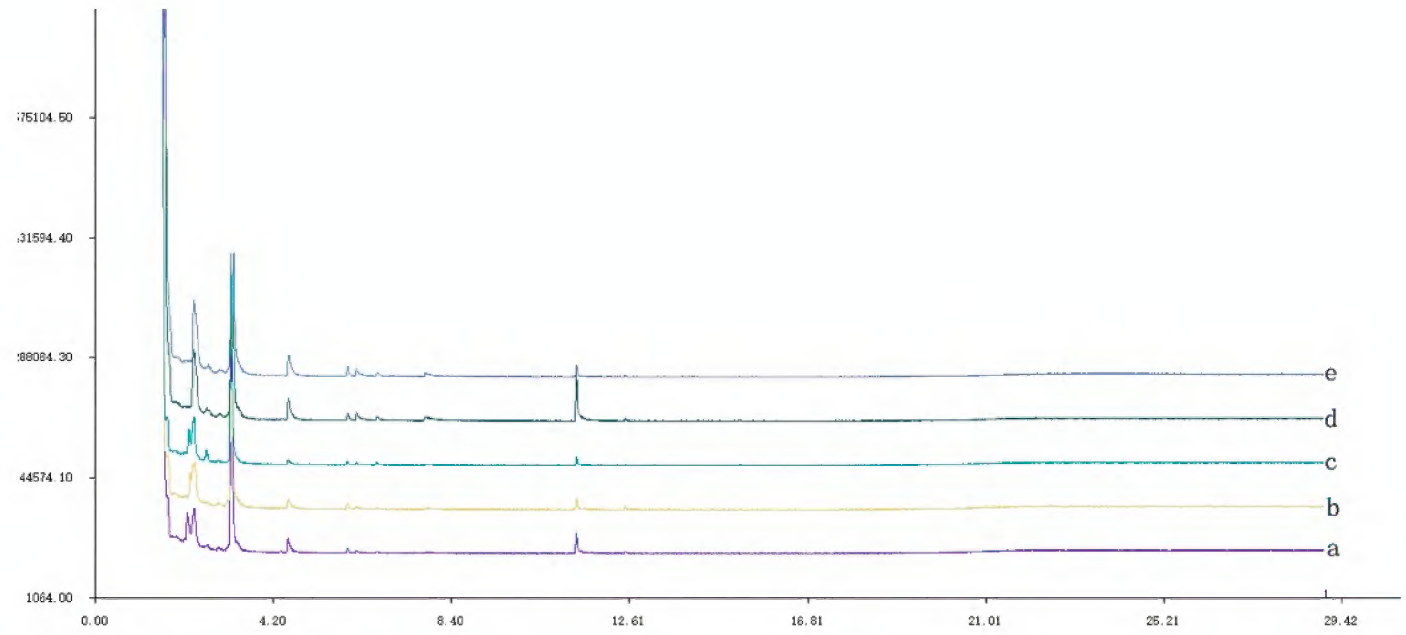

Figure 5. Total ion chromatogram of volatile components in flower at full-blossom **a** 8:00 **b** 10:00 **c** 12:00 **d** 14:00 **e** 16:00.

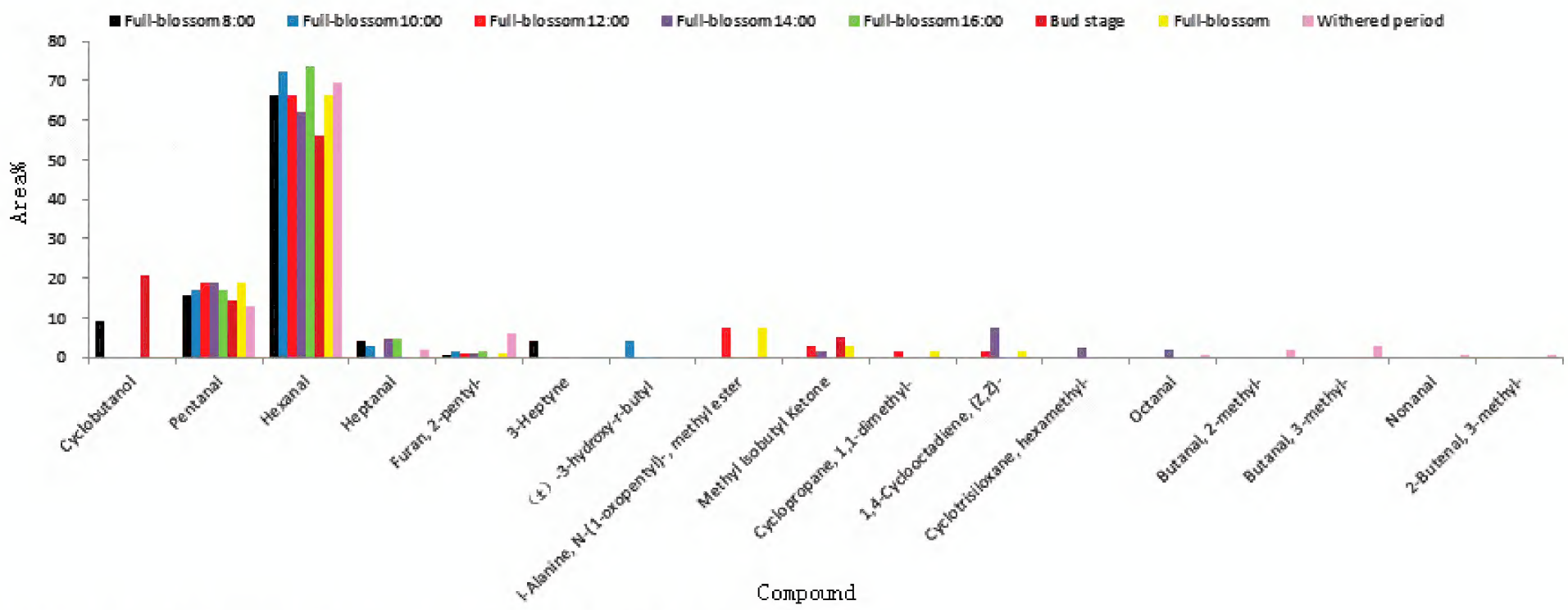

Figure 6. Variation of volatile components in different time periods.

In light of these observations, we conclude that *C. kanran* primarily relies on deceptive means to entice *A. cerana* for pollination, representing a fascinating example of an orchid species that has evolved unique strategies to ensure its reproductive success. Further research is warranted to elucidate the specific mechanisms underlying this deceptive pollination strategy in *C. kanran*.

Impact of deceptive pollination on *C. kanran*'s fruit set rate

The presence of deceptive pollination in *C. kanran* has a substantial impact on its fruit set rate. Our artificial pollination experiments provided valuable insights into the reproductive mechanisms of this orchid species. Specifically, the fruit set rate of bagged flowers that were not subjected to stamen removal was recorded at 0%, indicating the absence of apomixis in *C. kanran*. Fully bagged flowers also exhibited a fruit set rate of 0%, effectively ruling out automatic self-pollination. These findings underscore the critical reliance of *C. kanran* on pollinators for seed formation.

In natural conditions, non-rewarding orchids typically exhibit an average fruit set rate of approximately 27.7% (Jersakova et al. 2006). Intriguingly, the natural fruit set rate of *C. kanran* appears to be even lower. However, when subjected to artificial pollination, *C. kanran* demonstrated a remarkably high fruit set rate,

approaching 100%. This stark contrast between natural and artificial pollination outcomes strongly suggests that the reproductive success of *C. kanran* is significantly constrained by the availability and effectiveness of pollinators (Table 1).

It is well-documented that the fruit set rate of non-rewarding plants tends to be lower than that of rewarding plants. This phenomenon can be attributed, in part, to the learning abilities of pollinating insects, particularly in social species like *A. cerana*. Insects receiving deceptive signals from a plant, landing on floral organs devoid of rewards, are less likely to revisit similar flowers, reducing the frequency of visits to deceptive flowers. Furthermore, the success of pollination hinges on whether pollen-carrying pollinators can be deceived twice and return to the plant's flowers. Our observations indicate that after visiting non-rewarding flowers, most insects tend to avoid the population, diminishing the likelihood of a second deceptive encounter. Consequently, in many deceptive plants, the number of pollen outflows does not match the number of inflows (Johnson et al. 2003). As exemplified in this study, the number of pollinia outflows significantly exceeded the number of inflows (Fig. 2), highlighting the intricate dynamics of deceptive pollination in *C. kanran*.

Enhanced opportunities for outcrossing via deceptive pollination

In the context of deceptive pollination, it is worth noting that this strategy may have a positive influence on promoting outcrossing in plant populations, as exemplified in species like *C. kanran*. These plants often exhibit rhizomatous growth patterns and tend to form patches in their natural habitats. Within a given population, these patches typically consist of several clones of rhizomes.

In general, for clone-forming plants, as the size of the clone base expands, individual flowers become increasingly surrounded by other flowers originating from the same clone. This spatial proximity can facilitate the transfer of pollinia within the clone while impeding the dispersal of pollinia between different clones. This scenario inherently elevates the risk of self-pollination (Handel 1985). However, our observations of *C. kanran* introduce an intriguing twist to this dynamic. Since pollinators visiting *C. kanran* do not receive any rewards, they tend to depart from the population after attempting to visit only 1–5 flowers. This behavior significantly limits the extent of self-pollination within the clone, effectively reducing the proportion of self-pollination and concurrently increasing the likelihood of outcrossing events.

In this way, the deceptive pollination strategy employed by *C. kanran* may serve as a mechanism to counteract the potential negative consequences of spatial clustering in clone-based populations, ultimately enhancing genetic diversity and contributing to the plant's evolutionary success. Further research is warranted to explore the genetic consequences of this deceptive pollination strategy and its implications for the long-term viability of *C. kanran* populations.

Specialized pollination systems enhance reproduction in scattered plant populations

The reproductive success of plants with dispersed distribution can be significantly bolstered by the presence of specialized pollination systems. These systems are intricately linked to floral characteristics, pollinator behavior,

pollination mechanisms, and overall plant fitness (Huang and Guo 2000). Through field observations, we have established that *C. kanran*'s primary pollinator is *A. cerana*, with pollinia situated on the back of these bees. The dimensions of the entrance channel formed by the petals, labellum, and gynandrium of *C. kanran* precisely align with the average thorax height and body width of *A. cerana*, ensuring that only these bees can come into contact with the pollinia for effective pollination. This exclusive relationship indicates that *C. kanran* employs a classic specialized pollination system with a distinct pollinator.

Remarkably, *C. kanran* is not an exception among Cymbidium species when it comes to specialization. With the exception of *C. madidum* and *C. suave* (Davies et al. 2006), all other Cymbidium species, including *C. goeringii* and *C. floribundum*, also exhibit a preference for a single pollinator species (Yu et al. 2008). In the natural environment, *C. kanran* populations are dispersed over a wide area, presenting challenges for effective gene flow. Nevertheless, the specialized pollination system adapted by *C. kanran*, closely aligned with the behavior of *A. cerana*, enhances its reproductive success.

This strategic adaptation effectively overcomes the limitations imposed by scattered habitats. The synergy between the unique flower traits of *C. kanran* and the foraging habits of *A. cerana* facilitates efficient pollen transfer across considerable distances, mitigating the genetic isolation that could result from the species' fragmented habitat. Ultimately, this adaptation contributes to the maintenance of genetic diversity, enhances adaptive potential, and secures reproductive success. In essence, *C. kanran*'s specialized pollination strategy serves as a mechanism for overcoming the spatial challenges posed by its ecosystem, ensuring effective reproduction and the preservation of its genetic diversity pool (Jacome-Flores et al. 2019).

Some aldehyde compounds may be effective volatile compounds for attracting pollinators

Plants employ various deceptive strategies to attract pollinators without providing any tangible rewards. These strategies encompass generalized food deception, Batesian mimicry, mimicry of oviposition sites, sexual deception, and even the release of insect pheromones. In the first two strategies, plants typically rely on visual signals to lure pollinators. However, our Glass Cylinder experiment unequivocally demonstrated that *C. kanran* primarily employs olfactory signals rather than visual cues to entice pollinators, effectively excluding generalized food deception as a viable strategy (Handel 1985; Johnson et al. 2003; Ravigne et al. 2006).

Among the volatile compounds detected in our study, pentanal and hexanal consistently exhibited relatively high levels throughout the flowering period. Additionally, heptanal and 2-pentylfuran were absent during the bud stage but became present during the full bloom stage, with their levels displaying no discernible regular patterns. Furthermore, 4-methyl-2-pentanone and 1,4-cyclooctadiene emitted volatile odors during the period when insect visitation was most frequent, particularly from 12:00 to 14:00. Hence, our speculation centers on pentanal, hexanal, heptanal, 2-pentylfuran, 4-methyl-2-pentanone, and 1,4-cyclooctadiene as potential effective volatile compounds for attracting pollinators.

It is worth noting that hexanal has previously been demonstrated to play a pivotal role in attracting *A. cerana* to Jatropha curcas (Luo et al. 2013). Additionally, heptanal has displayed strong attraction to *A. cerana* and is frequently employed in proboscis extension reflex tests to assess the learning ability of these bees (Luo et al. 2022). These findings suggest that these aldehyde compounds may indeed serve as effective volatile compounds for alluring pollinators to *C. kanran* blossoms.

The role of flower colors in *C. kanran*'s pollination strategy

C. kanran exhibits a remarkable diversity of flower colors within the Cymbidium genus. However, behavioral experiments involving *A. cerana* have revealed that these flower colors do not hold significant attraction for these Chinese honeybees; instead, the primary allure lies in the plant's scent. This observation challenges the conventional belief that flower colors predominantly evolve to captivate pollinators, prompting inquiries into the evolutionary significance of *C. kanran*'s diverse flower palette.

Firstly, while behavioral experiments suggest that scent is paramount in attracting *A. cerana*, it is conceivable that the varied flower colors of *C. kanran* may still entice other pollinators. The species inhabits diverse ecological niches, each harboring its own spectrum of insect species. Consequently, the profusion of flower colors in *C. kanran* might cater to different pollinator preferences across distinct habitats, thereby increasing its chances of successful pollination (Kremen and Ricketts 2000; Olesen and Jordano 2002).

Secondly, the abundance of flower colors in *C. kanran* could be a testament to its evolutionary history. Throughout the evolutionary timeline of plants, flower colors might have served diverse functions in attracting pollinators, potentially at different times or under varying environmental conditions. Although current experimental evidence indicates that flower colors may not presently captivate pollinators, it does not necessarily negate their historical or future significance. The myriad flower colors of *C. kanran* may represent the outcome of genetic inheritance and intricate evolutionary processes in plants, with the possibility of these colors assuming distinct roles under varying environmental circumstances (Liu et al. 2014; Rudall 2020).

These insights into the interplay between flower colors and pollination strategies expand our understanding of the multifaceted mechanisms plants employ to ensure their reproductive success and adapt to dynamic ecological contexts.

Implications for conservation of *C. kanran*

Presently, all wild orchid species fall within the protective ambit of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, constituting a majority of the plant species safeguarded by this international convention.

Studying the pollination characteristics of plants not only reveals their survival strategies and ecological adaptability but also provides valuable insights for the conservation of rare and endangered species. Orchids, in particular, have evolved mutualistic relationships with their pollinating insects, with some forming specialized one-to-one pollination associations. The vulnerability of

these pollinating insects directly affects the outcomes and reproductive capabilities of the corresponding orchids, thereby influencing their overall survival (Aizen 2021).

This study found that *A. cerana* is the sole pollinator of *C. kanran*, and a decrease in its population may impact the reproductive capacity of *C. kanran*. The study also revealed that the natural fruit set rate is much lower than that achieved through artificial pollination, primarily due to the insufficient presence of Chuanpollia bees. Increasing the population of pollinators is advantageous for enhancing the fruit set rate. Firstly, the protection of wild *A. cerana* populations is crucial, and capturing wild bee colonies should be prohibited, especially given the current popularity of wild bee honey in the Chinese market, driving locals to capture wild bee colonies for profit. Secondly, planned releases of *A. cerana* should be conducted, allowing the new bee colonies to thrive in the natural environment without human interference. Thirdly, in areas where *C. kanran* is distributed, select a location with convenient flight paths for bees, close to water sources, away from agricultural orchards, with a mix of shade and sunlight. Construct simple beehives to attract bee colonies to settle in these areas. Fourthly, during the flowering period of *C. kanran*, encourage beekeepers to relocate their bee colonies to the areas where *C. kanran* is distributed (Theisen-Jones and Bienefeld 2016; Liu et al. 2022). Fifthly, plant some nectar-producing plants that bloom simultaneously with *C. kanran* to increase the frequency of *C. kanran* visits (Dellinger 2020). Additionally, the study found that *C. kanran* communities are dispersed, with some populations located far beyond the flight range of *A. cerana*. For populations distant by 4–6 km, consider artificially planting *C. kanran* in intermediate locations to connect the dispersed *C. kanran* communities, thereby increasing the probability of pollinators carrying pollen to the stigma (Finger et al. 2014; Dellinger 2020).

Furthermore, as *C. kanran* is extensively cultivated for its ornamental value, this research underscores its potential applications in artificial cultivation. Intervarietal hybridization typically involves manual pollination. Building upon the insights gleaned from this study, the introduction of *A. cerana* to *C. kanran* plantations or the cultivation of honey plants to draw in *A. cerana* for pollination could markedly elevate the fruit set rate. Moreover, the pollination mechanism orchestrated by *A. cerana* in *C. kanran* conduces to a heightened rate of cross-breeding, facilitating an increase in intervarietal hybridization. Consequently, this augments the prospect of breeding variants displaying a broader array of phenotypes. Such an approach also has the potential to mitigate illicit harvesting of *C. kanran* by unscrupulous flower farmers.

Conclusion

In sum, this study reveals that in its natural state, *C. kanran* must rely on pollinators for successful fruit setting, with *A. cerana* being the sole pollinator; however, the insufficient population of *A. cerana* leads to a lower fruit-setting rate in *C. kanran*. *C. kanran* does not offer rewards to pollinators but attracts them for pollination by releasing volatile compounds such as hexanal, heptanal, octanal, 2-pentyl furan, 4-methyl-2-pentanone. These research findings provide scientific guidance for the conservation of *C. kanran*, and corresponding conservation strategies have been proposed.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Luo and Xiao performed most of the experiments and data analysis. Liu, Chen, and Xiong participated in the experiments. Wu and Huang participated date analysis. Yang designed the experiments. Luo, Xiao and Wu prepared the manuscript. All authors read and approved the final version of the manuscript.

Author ORCIDs

Hanwen Xiao https://orcid.org/0000-0003-4527-696X
Xinchen Wu https://orcid.org/0009-0008-1221-0044

Data availability

All of the data that support the findings of this study are available in the main text.

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